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TITLE: Identification of prostate cancer related genes using inhibition of NMD in prostate cancer cell lines

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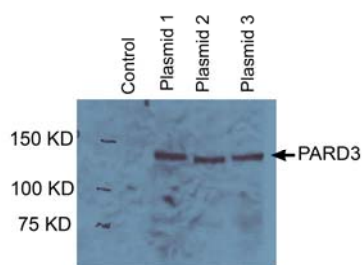
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14. ABSTRACT A strategy to identify mutant genes using inhibition of nonsense mediated decay (NMD) in cell lines has been proposed by others. Blocking translation with antibiotic emetine has been shown to inhibit the NMD. Stabilization of mutant mRNA following the inhibition of NMD with emetine can be detected using microarray technology, such as Affymetrix genechips, for example. Unfortunately, too many genes that do not contain any mutations show mRNA increase following emetine treatment due to stress response to the inhibition of translation or due to being a natural substrate for NMD, thus complicating the identification of mutant genes. We have developed a modification of the method which includes inhibition of NMD using two alternative methods. First is to inhibit translation with emetine. Second is to block NMD by inhibiting SMG-1 kinase with caffeine and then to block transcription with actinomycin D and either to continue blocking NMD with caffeine or to activate NMD by caffeine withdrawal. Analyzing the mRNA accumulation following emetine treatment as well as mRNA degradation following blocking of transcription in the presence or absence of NMD with the Affymetrix analysis using simple analytical algorithm allows selection of candidate genes for sequencing with high efficiency. Using our method of NMD inhibition we have identified several genes containing bi-allelic inactivating mutation in prostate cancer cell lines.					
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Table of Contents

	Page
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	4
Reportable Outcomes.....	5
Conclusions.....	5

ADDENDUM TO FINAL REPORT

Although the funding for functional studies had been cut in the original grant application, during the year 2007 we tried to express the wild type copies of the CLPTM1 and PARD3 under CMV promoter into LNCaP prostate cancer cell line where these genes had been found by us to contain bi-allelic inactivating frameshift mutations. We cloned the cDNA for these genes with attached FLAG tag into pcDNA3.1 vector from Invitrogen. Although we were able to transiently express proteins in the HEK293 cells as can be seen on the figure for the PARD3 gene we could not achieve stable expression of these genes in the LNCaP cells. It is possible that due to tumor suppressor capability of these genes there was a negative selection against the expression of PARD3 and CLPTM1 proteins in the LNCaP cells. Therefore we have changed the strategy and have generated the constructs for the expression of these genes under the tetracycline inducible promoter. Dr. Powell from the Cleveland Clinic has provided us with the LNGK-9 cell line, which is a LNCaP cells expressing the Tet-off regulatory plasmid for the inducible expression of genes. Unfortunately, we don't have any money left to continue the functional studies on these potential prostate tumor suppressor genes. I will be applying for new grant for the Department of Defense to continue studying of the role of the PARD3 and CLPTM1 genes in prostate carcinogenesis.



Western Blotting with anti-FLAG antibody shows the expression of the FLAG-tagged PARD3 protein in the 293 cells transiently transfected with the three different plasmids containing PARD3 cDNA insert.

SUMMARY STATUS OF TASKS OUTLINED IN THE STATEMENT OF WORK

Task1. Completed

Task2. Completed.

KEY RESEARCH ACCOMPLISHMENTS

- 1) An efficient method of selection of the candidate genes for sequencing analysis from the genes that show mRNA increase following inhibition of NMD has been developed.
- 2) An alternative version of GINI analysis which uses analysis of NMD mediated mRNA degradation in the absence of transcription following inhibition of NMD with caffeine has been developed.
- 3) Ten genes containing inactivating biallelic mutations in prostate cancer cell lines have been identified using our version of GINI and analytical algorithm to select genes for sequencing analysis.

REPORTABLE OUTCOMES

Publications

M. Rossi, L. Hawthorn, J. Platt, T. Burkhardt, J. Cowell, Y. Ionov. Identification of inactivating mutations in the JAK1, SYNJ2, and CLPTM1 genes in prostate cancer cells using inhibition of nonsense-mediated decay and microarray analysis. *Cancer Genetics and Cytogenetics* 2005, September 161(2):97-103

I Ivanov, K C Lo, L Hawthorn, J K Cowell and Y Ionov. Identifying candidate colon cancer tumor suppressor genes using inhibition of nonsense-mediated mRNA decay in colon cancer cells. *Oncogene*, 2007

CONCLUSIONS

Through the funded period we have developed a reliable method to identify mutant genes in prostate cancer cell lines using inhibition of NMD and Affymetrix GeneChip analysis. The efficiency of the method is close to 50%, which is the highest reported for the identifying mutant genes using GINI analysis. The approach which includes combining inhibition of NMD and comparative genomic hybridization, described by other group (Huusko et al (2004)) is much less efficient than the one developed in our lab. Analyzing several cell lines they were able to identify mutation only in one gene, EPHB2 in one cell line.. Ten genes have been identified to contain biallelic inactivating mutations in prostate cancer cell lines using our approach. The reason for this is that in prostate cancer cell lines with microsatellite instability, such as in LNCaP, DU145 and 22Rv1 inactivation of both alleles by two independent mutations is more likely than mutation in one allele and loss of the other. Therefore the addition of CGH analysis to GINI provides little advantage. Several genes previously not known to be mutated, such as CLPTM1, AS3, PARD3, are potential prostate tumor suppressor genes.